Report on the research cruise

EU FET Project: PROSPECTOMICS

Vessel: R/V H.U.Sverdrup II

Dates: 29.10.-5.11.2021

Ports: Tromsø-Tromsø

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Chief Scientist: Jens Kallmeyer, GFZ Potsdam

Expedition Project Manager: Petter Lagstad, FFI

1. Project objectives

The sole objective of the cruise was the collection of samples for the EU-funded FET (Future Emerging Technologies) project PROSPECTOMICS. The working hypothesis of the project is that all hydrocarbon reservoirs leak to some degree and that the upwards migrating hydrocarbons will cause a shift in microbial activity, abundance and community composition. Even if the hydrocarbons are fully mineralized during their ascent through the sediment and do not form any kind of surface manifestation, the microbial community will differ from those in sediments that are not affected by hydrocarbon leakage.

This project aims to develop a novel multi-omics-based approach to hydrocarbon exploration by analyzing the microbial community in near-surface sediments. The vast amount of omics data requires statistical evaluation by machine learning and given that all omics data are in-silico reconstructions, they need to be put into a geologic and geochemical context to derive any meaningful conclusions.

The project involves the several partners, which contribute specific expertise:

- GFZ Potsdam, Germany (GFZ): Project coordination, geochemical analyses, sample curation
- Universität Duisburg-Essen, Germany (UDE): Metagenomics
- Université du Luxembourg, Luxemburg (uni.lu): Metatranscriptomics
- Universität Greifswald, Germany (UG): Metaproteomics
- Universität Wien, Austria (VIE): Machine Learning
- Lundin Energy/Aker BP, Norway: Site selection, cruise planning, sample curation

2. Cruise summary

Based on the long-standing collaboration between FFI and Lundin Energy, the Research Vessel R/V Sverdrup II was chosen. All administrative tasks were handled by Lundin.

The cruise was scheduled to operate out of Tromsø, dates were set to October 29th to November 8th 2021. Due to Covid-19 restrictions in place at that time the science party was limited to five persons.

In order to have a suitable working environment GFZ brought the mobile geomicrobiology laboratory “BugLab”. This unit is housed in a standard 20’ shipping container and contains a fully outfitted microbiological laboratory, including ultralow (-80°C) freezer and a fume hood for handling hazardous chemicals. All sampling was carried out via gravity coring. The necessary equipment was provided by FFI.

After some preparations and trial runs of the corer while still in Tromsø harbour, the vessel left port on the evening of October 29th and reached the first sampling location next morning. Due to favorable weather conditions, sampling was uninterrupted from October 30th to November 5th, then the cruise had to be aborted two days early due to a medical emergency of one of the crew members. The ship was back in Tromsø on November 6th.

The work on board focused on recovery of the maximum number of gravity cores and included only minimal subsampling and no on-board analyses at all. See the methodology section for details. In total 50 gravity cores, ranging in length between ca. 1 and 2.3 m, were recovered.

Figure 1: Sampling operations on board the research vessel R/V Sverdrup. (**A**) The research vessel R/V Sverdrup, (**B**) crane and A-frame used to deploy the gravity corer, (**C**) gravity corer being lowered into the water, (**D**) sediment core retrieval, (**E**) deck operations starting with (**F**) core subsampling, (**G-H**) pore water extraction using rhizons, (**I**) subsampling for sulfate reduction rates in 0.5 m intervals, and (**J**) Buglab operations including (**K**) pore water aliquoting and (**L**) sample packaging in N_2 gas-tight aluminum foil bags sealed for storage at -80°C.

3. Participants

Chief Scientist, Principal Investigator

- Dr. Jens Kallmeyer, GFZ

Science Crew

- Ellen Schnabel, MSc., PhD student, GFZ
- Dipl. Ing. Jan Axel Kitte, Engineer, GFZ
- Steffen Okolski, Technician, GFZ
- Edgar Kutschera, MSc., Videographer, Lab Assistant, Frane Media

Operations Crew

- Petter Lagstad, Operations Manager, FFI
- Christian Ugelstad, Engineer, FFI
- Vidar Forsmo, Engineer, FFI

4. Research Program

4.1 Description of Working Area

Investigations were carried out in areas of former exploration license PL954 (block 7121/2). Gravity core positions within the study area were located in three zones with known positive hydrocarbon anomalies (Z1, Z10, Z12) and Reference zones (Ref5, Ref6). We aimed for about 50 m spacing between coring positions to assess spatial heterogeneity in each zone. However, at some prospective coring locations rocks or hard grounds were visible in photographs that were taken during ROV deployments during previous cruises. Such locations were spared, e.g. in the center of Zone 1.

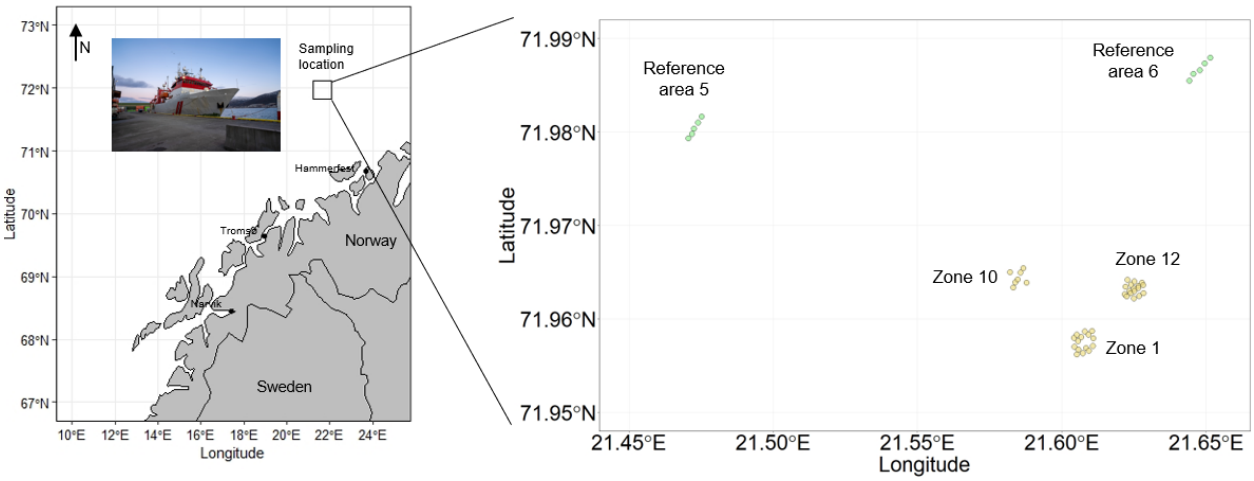

Figure 2. Sampling locations. The fifty cores were retrieved from three HC seepage zones (i.e. Zone 1, Zone 10 and Zone 12) and two reference zones (i.e. Ref 5 and Ref 6) without seepage.

Date	ID	Water	Position (degrees)		Position (decimals)		Core length	Remarks
		depth	Lat	Lon	Lat	Lon	cm	
30.10.21	Z1_C01	350m	71°57.47859N	021°36.25526E	71,957977	21,6042543	208	
30.10.21	Z1_C02	350m	71°57.49962N	021°36.31558E	71,958327	21,6052597	148	
30.10.21	Z1_C03	350m	71°57.41960N	021°36.26335E	71,956993	21,6043892	0	
30.10.21	Z1_C03	350m	71°57.42105N	021°36.26250E	71,957018	21,604375	133	
31.10.21	Z1_C04	352m	71°57.45889N	021°36.33762E	71,957018	21,605627	198	
31.10.21	Z1_C05	351m	71°57.48323N	021°36.40976E	71,958054	21,6068293	222	
31.10.21	Z1_C06	350m	71°57.51928N	021°36.48221E	71,958655	21,6080368	184	
31.10.21	Z1_C07	350m	71°57.37292N	021°36.31259E	71,956215	21,6052098	209	
31.10.21	Z1_C08	349m	71°57.40360N	021°36.34445E	71,956727	21,6057408	90	
31.10.21	Z1_C09	349m	71°57.50025N	021°36.56144E	71,958338	21,6093573	95	Bad weather
01.11.21	Z1_C10	351m	71°57.52315N	021°36.63177E	71,958719	21,6105295	85	
01.11.21	Z1_C11	350m	71°57.38052N	021°36.44300E	71,956342	21,6073833	160	
01.11.21	Z1_C12	350m	71°57.41368N	021°36.50425E	71,956895	21,6084042	189	
01.11.21	Z1_C13	350m	71°57.44132N	021°36.59526E	71,957355	21,609921	0	
01.11.21	Z1_C13	350m	71°57.44284N	021°36.58244E	71,957381	21,6097073	0	Missing upper seal
01.11.21	Z1_C14	350m	71°57.47496N	021°36.65578E	71,957916	21,6109297	114	Crust area?
01.11.21	Z1_C15	350m	71°57.39460N	021°36.56383E	71,956577	21,6093972	173	
01.11.21	Z1_C16	351m	71°57.42736N	021°36.64666E	71,957123	21,6107777	153	Replaced "closing system"
01.11.21	Z12_C01	347m	71°57.85070N	021°37.36615E	71,964178	21,6227692	155	

Date	ID	Water depth	Position (degrees) Lat	Lon	Position (decimals) Lat	Lon	Core length cm	Remarks
01.11.21	Z12_C02	348m	71°57.80687N	021°37.32654E	71,963448	21,622109	162	
01.11.21	Z12_C03	347m	71°57.82009N	021°37.44327E	71,963668	21,6240545	154	
01.11.21	Z12_C04	347m	71°57.84023N	021°37.51047E	71,964004	21,6251745	161	
02.11.21	Z12_C05	348m	71°57.75894N	021°37.30926E	71,962649	21,621821	161	
02.11.21	Z12_C06	348m	71°57.77914N	021°37.41113E	71,962986	21,6235188	170	
02.11.21	Z12_C07	348m	71°57.79508N	021°37.50236E	71,963251	21,6250393	162	
02.11.21	Z12_C08	347m	71°57.81296N	021°37.59202E	71,963549	21,6265337	179	
02.11.21	Z12_C09	346m	71°57.83231N	021°37.67270E	71,963872	21,6278783	200	
02.11.21	Z12_C10	348m	71°57.74481N	021°37.35087E	71,962414	21,6225145	165	
02.11.21	Z12_C11	348m	71°57.76584N	021°37.43145E	71,962763	21,6238575	169	
02.11.21	Z12_C12	347m	71°57.77843N	021°37.51183E	71,962974	21,6251972	170	
02.11.21	Z12_C13	347m	71°57.79853N	021°37.59692E	71,963309	21,6266153	167	
02.11.21	Z12_C14	347m	71°57.81915N	021°37.68386E	71,963653	21,6280643	0	
02.11.21	Z12_C14	347m	71°57.81739N	021°37.69123E	71,963623	21,6281872	137	
03.11.21	Z12_C15	347m	71°57.73308N	021°37.50337E	71,962218	21,6250562	195	
03.11.21	Z12_C16	347m	71°57.74841N	021°37.60842E	71,962474	21,626807	199	
03.11.21	Z12_C17	347m	71°57.76592N	021°37.70298E	71,996099	21,628383	116	
03.11.21	Ref6_C06	356m	71°59.12795N	021°38.65338E	71,985466	21,644223	56	
03.11.21	Ref6_C07	355m	71°59.17334N	021°38.73617E	71,986222	21,6456028	159	
03.11.21	Ref6_C08	355m	71°59.19635N	021°38.87095E	71,986606	21,6478492	160	
03.11.21	Ref6_C09	355m	71°59.23915N	021°38.97035E	71,987319	21,6495058	130	
03.11.21	Ref6_C10	355m	71°59.27758N	021°39.09079E	71,98796	21,6515132	128	
03.11.21	Ref5_C01	348m	71°58.75077N	021°28.24275E	71,97933	21,4707125	155	
03.11.21	Ref5_C02	348m	71°58.78830N	021°28.31769E	71,979805	21,4719615	134	
04.11.21	Ref5_C03	348m	71°58.82134N	021°28.35610E	71,980356	21,4726017	148	
04.11.21	Ref5_C04	348m	71°58.86061N	021°28.44408E	71,98101	21,474068	151	
04.11.21	Ref5_C05	348m	71°58.89980N	021°28.51781E	71,981663	21,4752968	166	
05.11.21	Z10_C09	350m	71°57.85351N	021°35.08494E	71,964225	21,584749	192	
05.11.21	Z10_C07	351m	71°57.80259N	021°34.99081E	71,963377	21,5831802	169	
05.11.21	Z10_C08	350m	71°57.83648N	021°35.04001E	71,963941	21,5840002	151	
05.11.21	Z10_C10	348m	71°57.89829N	021°35.14865E	71,964972	21,5858108	142	
05.11.21	Z10_C11	349m	71°57.92611N	021°35.20491E	71,965435	21,5867485	159	
05.11.21	Z10_C01	350m	71°57.90006N	021°34.92920E	71,965001	21,5821533	150	
05.11.21	Z10_C04	350m	71°57.88407N	021°35.01752E	71,96475	21,5836253	0	Missing liner…
05.11.21	Z10_C04	350m	71°57.88483N	021°35.03753E	71,964747	21,5839588	0	Pebbles
05.11.21	Z10_C14	350m	71°57.85419N	021°35.20389E	71,964237	21,5867315	66	
05.11.21	Z10_C17	351m	71°57.83287N	021°35.26951E	71,963881	21,5878252	181	

Table 1. Sampling locations. All 50 locations with position, water depth and core length. Most cores were between 1 and 2 meters long. The overall success rate was good, the only

4.2 Sampling and sample processing

The sole objective of the cruise was to recover as many gravity cores as possible and subsample them for later analyses in the home labs of the PROSPECTOMICS team members. On-board sample processing and analyses were kept to an absolute minimum. The core material consists of homogeneous grey clay, with sporadic occurrence of fine gravel <0.5 cm in diameter. When exposed to air, the colour of the sediment turns from grey to brownish, indicating that the sediment comes from an anoxic environment and that iron rapidly oxidizes when exposed to ambient conditions.

4.2.1 Sampling for molecular biology

Once the core was on deck, the bottom 20 cm were immediately cut off, capped and brought into the BugLab container for subsampling. The sediment was pushed out of the liner onto a sheet of flame-sterilized aluminum foil and the outer 5-10 mm of sediment were scraped off to remove possible contamination from surficial sediments. The trimmed piece of sediment was then transfered onto a new sheet of sterilized aluminum foil and cut into five aliquots for Metagenomics, Metatranscriptomics, Metaproteomics, Geochemistry and Backup. Each aliquot was packed into gas-tight metal-coated plastic foil sleeves, the ends were heat sealed. Each bag was then tree times evacuated and flushed with nitrogen gas to avoid any oxidation. All samples were then stored in a -80°C freezer.
Upon arrival in Tromsø the samples were transferred into insulated boxes filled with dry ice and shipped to the respective home labs via courier.

4.2.2 Pore water sampling

The remainder of the gravity core was cut into 1 m-long sections, capped and stored on deck for pore water extraction. Rhizon®samplers (Rhizosphere Research Products) were inserted into the sediment every 10 cm through a 4 mm holes that was drilled through the liner. We attached a 20 ml plastic Syringe and applied a vacuum for at least 12 hours. This was normally sufficient to extract at least 10 ml of pore water, although in some cases it took up to 24 hours to get a sufficient amount. A total of 505 individual pore water samples were recovered, which were then aliquoted and preserved for the various analyses. To avoid additional steps, the pore water was directly dispensed from the syringe, which was fitted with an 0.2 µm filter.

- Alkalinity: Pore water was carefully dispensed into a 1.7 ml glass vial pre-loaded with 100 µL of saturated Mercuric Chloride solution. The vial was filled to the very top and closed with a septum screw cap without any headspace. Samples were stored at room temperature
- Anions: 2 ml of pore water were put into a 3 ml screw cap plastic vial and stored at +4°C.
- Cations: 2 ml of pore water were put into a 3 ml screw cap plastic vial pre-loaded with 20 µL of concentrated suprapure Nitric acid. Samples were stored at +4°C
- Sulfide: 2 ml of pore water were put into a 3 ml screw cap plastic vial pre-loaded with 100 µL of 20% Zinc Chloride solution. Samples were stored at room temperature.
- Backup: Depending on the amount the remainder of the pore water was stored in a 3 mL or 15 mL centrifuge vial and stored at +4°C.

All analyses were carried out in the home lab.

4.2.3 Sulfate reduction rates

Sulfate reduction is the quantitatively most important anaerobic process in the degradation of organic matter in anoxic marine sediments. In this process, sulfate is reduced to sulfide, which can remain in solution as H_2S or precipitate with metal ions as mono- or disulfides (e.g. mackinawite as FeS, pyrite as FeS_2). A minor fraction can also be found as elemental sulfur. In order to determine sulfate reduction rates, the sediment samples were spiked with sulfate radiotracer and incubated.

Structurally intact sediment samples of about 5 cm^3 were removed from the cut ends of the core sections with glass barrels fitted with a syringe plunger. Once the sediment sample was retrieved, the open end was closed with a butyl rubber stopper. Glass barrels are preferred over cut-off syringes as they provide a gas-tight enclosure.

The sediment samples taken during the day are stored in the fridge in the BugLab container until the evening, after deck work has stopped. Each sample is then injected with 15 µl of $^{35}SO_4$-Radiotracer (ca. 200 kBq), according to the whole-core injection technique of Jørgensen (1978) and incubated for 24 hours. Incubation was stopped by pushing the sediment into centrifuge vials, pre-filled with 15 ml of 20% (w/v) zinc acetate solution. The vial was thoroughly shaken to break up all sediment clumps, quickly stop all microbial activity and precipitate all sulfide as zinc sulfide. Samples were stored frozen at -20°C until analysis. Chemical separation of the produced radioactive sulfide species was carried out in the home lab, using the cold chromium distillation technique of Kallmeyer et al. (2004).

4.2.4 Cell counts

Like for Sulfate reduction rates, samples were taken from the freshly cut ends of the core sections. A sterile cut-off 3 ml syringe is inserted into the sediment and exactly 2 ml are pushed out and into a 15 ml centrifuge tube, pre-filled with 8 ml of a solution, containing 20 g/L NaCl and 2 vol% Formalin. Vials are shaken to break up sediment clumps and stored at +4°C. Cell enumeration via staining with CYBR Green I and fluorescence microscopy was carried out in the home lab.

5. Video documentation

As part of our outreach activities in the FET Project, we hired a videographer, Edgar Kutschera of Frane Media. He accompanied us on the cruise and produced a 45-minute documentary about our project in general and the cruise in particular. The video can be found on Youtube (https://www.youtube.com/watch?v=T_jGxauOEiQ).

6. Concluding remarks

Thanks to a fortuitous combination of unusually good weather and smooth collaboration between Crew, FFI staff and the Science Party, all cruise objectives could be met, despite the cruise being cut short by two days due to a medical emergency. Analysis of the collected samples is underway, preliminary results were already presented at various conferences.

7. Presentations

Esser S., Soares A., Kunath B.J., Laczny C.C., Schnabel E., Kitte J.A., Okolski S., Kutschera E., Kallmeyer J., Vuillemin A., di Primio R., Probst A.J. (2023) Taxonomic and functional diversity of microbiomes across hydrocarbon-affected and non-affected sites in the Barents Sea. 9th International Symposium on Applied Microbiology and Molecular Biology in Oil Systems (ISMOS), Edinburgh, June 27th-30th.

Kallmeyer J. (2023). Using molecular biological techniques for hydrocarbon prospecting – The PROSPECTOMICS Project. 1st Energy Geoscience Conference (EGC1), Aberdeen, May 16th-18th.

Kallmeyer J. (2023). Using molecular biological techniques for hydrocarbon prospecting – The PROSPECTOMICS Project. International Symposium on Applied Microbiology and Molecular Biology in Oil Systems (ISMOS), Edinburgh, June 27th-30th.

Ostrzinski A., Soares A.R., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2021). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. 4th International Metaproteomics Symposium, Luxembourg, September 27th – 29th 2021.

Ostrzinski A., Soares A.R., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2022). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. Proteomic Forum EuPA, Leipzig, April 3rd-7th.

Ostrzinski A., Soares A.R., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2022). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. EMBO practical course "Integrated multi-omics analyses of microbial communities, Luxemburg, April 23rd-29th.

Ostrzinski A., Soares A.R., Kunath B.J., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2023). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. 5th International Metaproteomics Symposium, Avignon, April 25th-27th.

Ostrzinski A., Soares A.R., Kunath B.J., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2023). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. 9th International Symposium on Applied Microbiology and Molecular Biology in Oil Systems (ISMOS), Edinburgh, June 27th-30th.

Ostrzinski A., Soares A.R., Kunath B.J., Laczny C.C., Kallmeyer J., di Primio R., Probst A.J., Trautwein-Schult A., Becher D. (2023). Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation. FEMS Conference, Hamburg, July 9th-13th.

Pfundner A., Rattei T. (2023) Adaptive machine learning for heterogeneous multi-omics data in a prolonged incremental data acquisition environment. 18th Machine Learning in Computational Biology (MLCB2023), poster presentation, Seattle, USA, November 30th-December 1st.

Schnabel E., Kallmeyer J. (2022). Detection of minor hydrocarbon seepage in marine sediments via sulfate reduction measurements. ISME18 Conference, Lausanne, August 14th-19th (poster).

Schnabel E., Kallmeyer J. (2023). Geochemical detection of minor hydrocarbon seepage in marine sediments. EGU23 General Assembly 2023, Vienna, April 23th -28th.

Schnabel E., Kallmeyer J. (2023). Geochemical detection of hydrocarbon reservoirs from marine surface sediments. 1st Energy Geoscience Conference (EGC1), Aberdeen, May 16th-18th.

Schnabel E., Kallmeyer J. (2023) Geochemical detection of minor hydrocarbon seepage in marine subsurface sediments. GeoBerlin, oral presentation, Berlin, Germany, September 3rd-8th.

Schnabel E., Jørgensen S.L., Kallmeyer J. (2023) Effect of minor hydrocarbon seepage on microbial turnover of nitrogen compounds.International Center for Deep Life Investigation (IC-DLI) Conference, Sanya, China, October 23rd-26th.

Soares A., Kaspareit Y., Kallmeyer J., Schnabel E., Kitte J.A., Okolski S., Kutschera E., Probst A.J. (2022). Capturing virus-host interactions via long-read Hi-C metagenomics. ISME18 Conference, Lausanne, August 14th-19th.